Results: The resorption pits made by osteoclasts were well demonstrated on the cortical bone plates. The area of the resorption pits became larger sequentially on day 3, 5, and 7 in osteoclast-cell culture. In the STZ group, the resorption-pit area was significantly decreased compared with that in control group. The expression levels of MMP9, Catepsin-K, and RANK showed no difference in between control and STZ group. On the other hand, expression levels of DC-STAMP were significantly decreased in STZ group.
Conclusions: In the last meeting, we reported that the osteoclast fusion was impaired, the cartilage resorption was decreased, and the endocondral ossification was delayed in the STZ-induced diabetic mice. In this study, we found that the impaired bone resorption in STZ might be produced by a decreased expression of DC-STAMP in osteoclasts. Since DC-STAMP has a central role for the formation of multinuclear osteoclasts, our result indicates that the up-regulation of DC-STAMP may be useful to prevent osteopenia in diabetes mellitus.

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COMPRESSION INDUCES THE EXPRESSION OF A SCLEROTIC PHENOTYPE IN HUMAN SUBCHONDRAL OSTEOBLASTS

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Purpose: Recent data showed that subchondral bone plays an important role in osteoarthritis (OA). Metabolic and morphologic modifications in this tissue contribute to the degradation of the overlaying cartilage. It was suggested that abnormal mechanical pressure applied to the articulation was responsible to these changes. Here, we evaluated the effects of compression on osteoblasts from subchondral bone.
Methods: Osteoblasts were isolated from sclerotic (SC) or non-sclerotic (NSC) areas of human OA subchondral bone. After 28 days, osteoblasts were surrounded by a newly synthesized matrix and formed a strong membrane. This osteoblasts-containing membrane was then placed onto a Biopress Flexercell plate and submitted to compression (1.67 MPa) for 4 hours at the frequency of 1 Hz. The expression of IL-6, IL-8, COX-2, VEGF, IGF-1, OPG and RANKL was evaluated by RT-PCR. IL-6, IL-8 and PGE2 were quantified by ELISA.
Results: Basal IL-6, VEGF, COX-2, IGF-1 and RANKL mRNA levels were significantly increased in SC osteoblasts as compared to NSC. By contrast, SC osteoblasts expressed less OPG than those from NSC areas. Compressions induced the expression of genes coding for IL-6, IL-8, COX-2, IGF-1, VEGF and RANKL but decreased the expression of OPG in NSC osteoblasts ($p<0.01$). IL-6, IL-8 and PGE2 productions were also stimulated by compressions. Interestingly, compressed NSC osteoblasts expressed similar levels of these genes than SC osteoblasts, suggesting that mechanical strains could be responsible for SC phenotype.
Conclusions: These results indicate that in response to compression NSC osteoblasts expressed a phenotype similar to that of SC osteoblasts. Moreover, SC osteoblasts are less sensitive to mechanical stimuli than NSC osteoblasts. These results clarify the role of compression in the pathogenesis of subchondral bone sclerosis and allow new perspectives of research in this field.

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INVOLVEMENT OF CCL20 CHEMOKINE IN BONE TISSUE REMODELING: EVIDENCE IN SUBCHONDRAL BONE OF OSTEOARTHRITIS AND RHEUMATOID ARTHRITIS PATIENTS

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Purpose: Bone tissues are remodeled through cycling of bone resorption and new bone formation that are dependent by the activity of osteoblasts and osteoclasts. Subchondral bone remodelling in osteoarthritis (OA) and rheumatoid arthritis (RA) is mainly characterized by the formation of osteophytes/fibrosis and by the presence of infiltrating cells associated to bone resorption. In this study we analysed on subchondral bone of OA and RA patients both the expression of CCL20 chemokine and its receptor CCR6 on osteoblasts, osteoclasts, osteocytes and infiltrating mononuclear cells. Then we analysed its effects on osteoblasts and osteoclasts.
Methods: CCL20 and CCR6 expression was evaluated in subchondral bone tissue biopsies by immunohistochemical techniques and the percentage of positive cells was manually counted. Functional assays (cell proliferation, matrix proteins expression, apoptotic markers, signalling factor and osteoclast differentiation) on osteoblasts and osteoclasts were performed to assess the functional role of CCL20.
Results: CCL20 was positive on osteoblasts (60%) and osteocytes (20%) from RA patients, while it was expressed only in OA osteoblasts located in area of new bone formation. Both in OA and RA biopsies, osteoclasts were highly positive to CCL20 while mononuclear cells were 10% positive in OA and 60% in RA. The percentage of osteoblasts positive to CCR6 was not different in OA versus RA biopsies, while the percentage of osteocytes and mononuclear cells positive to CCR6 was significantly higher in RA compared to OA. CCL20 did not affected both the expression of different matrix proteins (i.e. bone sialoprotein, collagen type I, osteopontin and osteocalcin) and apoptotic markers (like caspases 3 and 8) in OA and RA osteoblasts. CCL20-stimulated OA osteoblasts showed a significant increase in β-N-acetylhexosaminidase release compared to RA while osteoblasts proliferation was higher only in CCL20-stimulated RA osteoblasts associated to Akt phosphorylation. IL1β and TNFα differently modulated CCL20, RANKL/OPG expression in OA and RA osteoblasts. Moreover, we found that CCL20 was an early inducer (at day3) of cell fusion events, osteoclasts differentiation and MMP-9 release.
Conclusions: This study demonstrates a different expression of CCL20 positive osteoblasts in OA versus RA disease that seems to be associated with the presence of infiltrating mononuclear cells. Moreover, the contemporary action of CCL20 on osteoblasts and osteoclasts that resulted in a greater proliferative response in RA osteoblasts compared to OA and an increased enzymatic activity in OA osteoblasts, clearly suggests a differential role of this chemokine in OA and RA.

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NUCLEAR RECEPTOR RETINOID-RELATED ORPHAN RECEPTOR ALPHA1 MODULATES OSTEOBLAST METABOLISM WITH ANTI-INFLAMMATORY EFFECTS

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Purpose: To elucidate the expression and function of nuclear receptor retinoid-related orphan receptor alpha1 (RORalpha1) in human MG-63 osteoblast-like cells.